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Hesperomyces virescens (Fungi, Ascomycota, Laboulbeniales) attacking *Harmonia axyridis* (Coleoptera, Coccinellidae) in its native range

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Abstract This study intended to find data on obligate ectoparasitic Laboulbeniales (Fungi, Ascomycota) on Chinese *Harmonia axyridis* (Coleoptera, Coccinellidae). After having screened over four thousand dried specimens of *H. axyridis* and close relatives, we present the first (historical) record of Chinese *H. axyridis* infected with *Hesperomyces virescens*. We suggest that *H. virescens* is a historically globally distributed species and hypothesize that (native) infection was lost when *H. axyridis* was introduced in North America.

Keywords Coccinellidae · Distribution · Ectoparasitic fungi · *Harmonia axyridis* · *Hesperomyces virescens* · Historical data · Invasive species · Native range

1 Introduction

Invasive species may drive native species to extinction by mating with closely related native species (hybridization), or through competition for resources or space, a more efficient use of available food resources, predation, and indirect effects [1, 2]. Invasive “alien” species are now considered

one of the top drivers of global biodiversity loss. A striking example of a deliberate introduction that has gone out of control is that of the multicolored Asian ladybird *Harmonia axyridis* (Pallas, 1773) (Coleoptera, Coccinellidae). *H. axyridis* is native to Asia between Siberia at the North and Southern China at the South, and from the Altai Mountains to the Pacific Ocean including Korea and Japan [3]. It was first introduced in North America in 1916 for biological control of agricultural pests such as aphids and scale insects on crops including citrus, maize, and tobacco [4, 5]. It has been reported in all but two US states [6]. In western Europe, *H. axyridis* was first introduced in 1982 in France and is now considered established in 26 European countries [6].

Many theories have been put forward to explain the success of invasive species. The enemy release hypothesis predicts that an invasive species in its new environment will experience a decrease in regulation by natural enemies, resulting in a competitive advantage for the alien species over native species [7, 8]. While it has been suggested [9] that there is no support of natural enemies exerting regulatory effects on coccinellid populations, a recent study provides an overview of parasites and pathogens with focus on the resilience of *H. axyridis* compared to other Coccinellidae [10].

Here, we consider the obligate ectoparasitic fungus *Hesperomyces virescens* Thaxt. (Ascomycota, Laboulbeniales). Previous work states that *H. virescens* does not attack *H. axyridis* in its native range [10, 11]. We explore evidence available for assessing the (historical) distribution of *H. virescens*.

2 Materials and methods

Museum collections were examined for Laboulbeniales on *H. axyridis*, and close relatives that were selected based on recent

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phylogenies [12]: over four thousand dried specimens between 1915 and present were screened. The inspected entomological collections are deposited at the following institutions: National Museum of Natural History, Smithsonian Institution (1,274 specimens); Museum of Comparative Zoology, Harvard University (723 specimens); American Museum of Natural History (684 specimens); Field Museum (Chicago) (554 specimens); Department of Zoology, University of New Hampshire (513 specimens); Museum of Zoology, University of Michigan (314 specimens).

Parasitic thalli were carefully removed from the host integument using a BioQuip Minutem Pin and embedded in Amann solution [13]. Cover slips were ringed with transparent nail varnish. Morphological analyses and measurements were made using an Olympus BX40 light microscope with Olympus XC50 digital camera and MicroSuite Special Edition software 3.1 (Soft Imaging Solutions GmbH). Photography was done at the Harvard Center for Biological Imaging using a Zeiss AxioImager microscope running on ZEN software.

Microscopic slides are deposited at the United States National Fungus Collections (BPI) or at the Farlow Herbarium (FH), Harvard University.

3 Results

Of particular interest for this study was the discovery of Laboulbeniales parasitizing Chinese *H. axyridis*. Additional data resulting from the examination of the aforementioned entomological collections will be presented in subsequent papers.

A total of 1,152 *H. axyridis* of 4,062 studied pinned ladybirds were listed, 336 of which had been collected in China. They were distributed among the Museum of Comparative Zoology, Harvard University (173 specimens); the National Museum of Natural History (159 specimens); and the Museum of Zoology, University of Michigan (4 specimens). From these examined specimens only two specimens, deposited at the National Museum of Natural History, were found infected with *H. virescens*.

4 Taxonomy

Hesperomyces virescens Thaxt., Proc Amer Acad Arts Sci 1891, 25: 264 (Fig. 1).

= *Stigmatomyces virescens* (Thaxt.) Thaxt., Proc Amer Acad Arts Sci 1894, 29: 106.

4.1 Known distribution and hosts

Described from California (as *S. virescens*) on *Chilocorus stigma* (Say 1835) (as *Chilocorus bivulnerus*; Coleoptera,

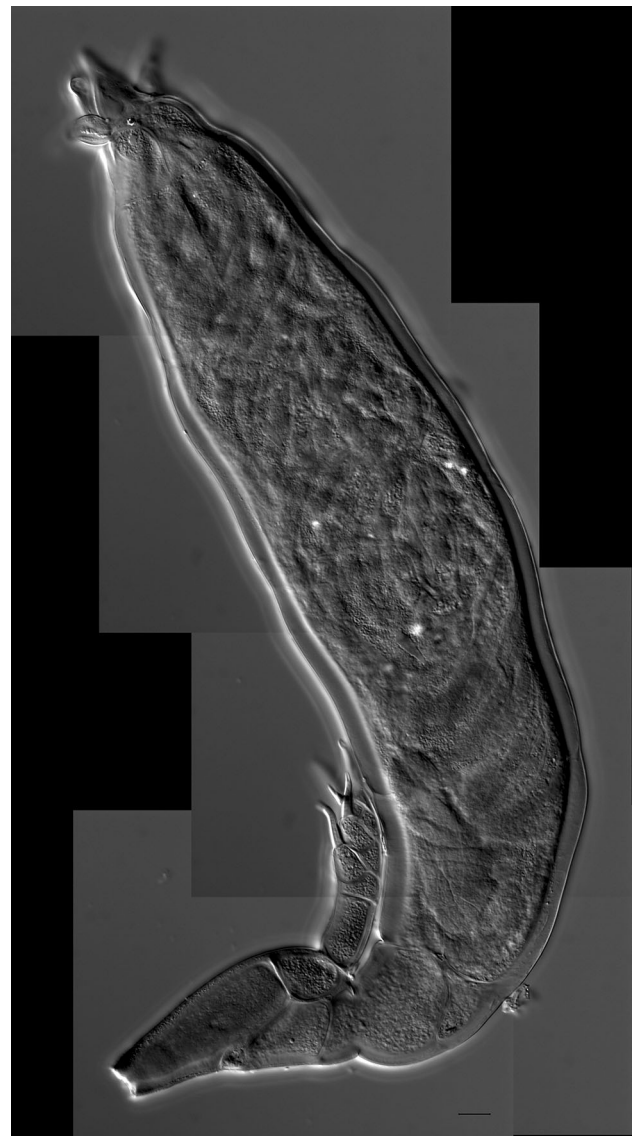


Fig. 1 *Hesperomyces virescens* Thaxt., mature thallus from left elytron of Chinese *Harmonia axyridis* (D. Haelew. 157a)

Coccinellidae), *H. virescens* is a widespread species known from all continents except Antarctica, attacking several coccinellid genera [14, 15]. It has only been reported upon *H. axyridis* in the USA, Belgium, the Netherlands, Germany, and the UK; a detailed summary with all recordings of *H. virescens* on *H. axyridis* so far is given in Table 1. In *H. axyridis*'s native range, more specifically in Japan, *H. virescens* is reported parasitizing *Coccinula crotchii* (Lewis 1879) and *C. sinensis* (Weise 1889) [32].

4.2 New chinese records of *H. virescens* on *H. axyridis*

CHINA, Sichuan, Yibin, 10–14 Apr. 1930, leg. D.C. Graham, # D.Haelew. 159, permanent slides D.Haelew. 159a (five specimens), 159b (three specimens), 159c (two

Table 1 All published records of *Hesperomyces virescens* on *Harmonia axyridis* (adapted from [16])

Region	Country	Year(s) collected	Reference
Sichuan Province	China	1930–1939	Present study
Wayne County, Ohio	USA	2002	[17]
Lebanon County, Pennsylvania	USA	2002–2003	[18]
Oktibbeha County, Mississippi	USA	2003–2004	[19]
North Carolina	USA	2003–2004	[20]
Harrison County, Kentucky	USA	2004	[21]
Washington County, Arkansas	USA	2005	[22]
Kentucky	USA	2005	[22]
Payne County, Oklahoma	USA	2005	[22]
Centre County, Pennsylvania	USA	2005	[22]
Brookings County, South Dakota	USA	2005	[22]
West Virginia	USA	2005	[22]
Meise, Flemish Region	Belgium	2006–2007	[23]
Peach County, Georgia	USA	2007	[24]
Western Mississippi	USA	2007–2009	[25]
De Kaaistoep, North Brabant	The Netherlands	2008	[26]
Hessia	Germany	2008–2009	[27]
De Kaaistoep, North Brabant	The Netherlands	2008–2011	[16]
Hohenstein, Hessia	Germany	2009	[28]
De Kaaistoep, North Brabant	The Netherlands	2009	[29]
Zundert, North Brabant	The Netherlands	2009	[16]
Ukkel, Brussels	Belgium	2010	[23]
Giessen, Hessia	Germany	2010	[11]
Peach County, Georgia	USA	2009	[30]
Flemish Region	Belgium	2011	[23]
De Kaaistoep, North Brabant	The Netherlands	2011	[31]
Utrecht	The Netherlands	2011	[31]
South East England	UK	2011	Present study
Tilburg, North Brabant	The Netherlands	2011	Present study
South Bohemian Region	Czech Republic	2013	[11]
Destelbergen, Flemish Region	Belgium	2013	Present study

specimens), and 159d (three specimens)—all specimens collected from distolateral part of right elytron, in coll. BPI; Sichuan, no date, leg. D.C. Graham, # D.Haelew. 157, permanent slide D.Haelew. 157a (three specimens collected from distomedian tip of left elytron), in coll. BPI.

4.3 Remark

The specimen # D.Haelew. 157 is labeled with collector (D.C. Graham) and location (Sichuan, China), but without a date. From Graham's reconstructed field itinerary, we can infer that he collected ladybird specimens in the Sichuan region during the period from 1919 to 1939. Within this timeframe, Graham completed fourteen summer expeditions to China, partly funded by the Smithsonian Institute

United States National Museum. Until 1948 he was affiliated with the West China Union University in Chengdu, capital of Sichuan province; afterwards he returned to the United States. Thus, any specimen collected by Graham in China most probably was collected during the period from 1919 to 1939, in any case before 1948.

5 Discussion

Roy et al. [10] suggest that *H. virescens* is probably native to North America, referring to the fact that Thaxter [33] first found it in the USA on *C. stigma* and *Hippodamia convergens* Guérin-Ménéville, 1842, both native to North America [34]; soon more recordings from new localities

extended the distribution of *H. virescens* to South America, Asia, and Oceania [35, 36]. The (historical) records for China of *H. virescens* parasitizing *H. axyridis* in this study give rise to the suggestion that *H. virescens* is a global species.

Although the first introduction of *H. axyridis* (of Japanese origin) in the USA happened in 1916 and despite at least fourteen reintroductions [4, 6], the first established population was only found in 1988, in Abita Springs, Louisiana [5]. Thereafter the species spread rapidly and currently is reported in all US states except for Alaska and Wyoming [6]. Microsatellite and mtDNA data show that the genetic structure in *H. axyridis* in its native range falls into two groups, east and west, and that populations from both groups have contributed to the invasive, so-called *bridgehead* population in eastern North America [4, 6, 37]. This combination between genetically diverse populations from the native range may have facilitated the success of *H. axyridis* in North America, and consequently its subsequent expansion. It has been suggested that with the rapid expansion of *H. axyridis* also *H. virescens* will continue to spread globally [10] and threaten native, noninvasive Asian populations of *H. axyridis* [11].

While *H. axyridis* was already present in the USA in 1916, the earliest infected North American *H. axyridis* was found only during the summer of 2002 (in Ohio) [17], 86 years after the first introduction and 14 years after the first established population in the USA. Based on the presented records, we consider *H. virescens* a historically globally distributed species that also attacks *H. axyridis* in its native range. Furthermore, we hypothesize that native *H. virescens* infection was lost when beetles were imported into North America, for example, because sampled specimens were “clean” (free from infection) [37], and in line with the natural enemy release hypothesis [7, 8]. After a time lag reinfection with local *H. virescens* occurred, the first evidence of this was recorded in 2002 [17].

6 Miscellaneous records of *H. virescens*

Micronesia, Mariana Islands, Agrihan Volcano, 17 Jul. 1949, leg. D.B. Langford, on *Harmonia octomaculata* (Fabricius 1781), permanent slides D.Haelew. 160a–h (81 specimens, all collected from the elytra), in coll. BPI. USA, Alaska, Matanuska Valley, 27 Jul. 1944, leg. J.C. Chamberlin, on *Hippodamia tredecimpunctata* (Linnaeus 1758), permanent slides D.Haelew. 163a (ten specimens collected from distomedian tip left elytron), 163b (seven specimens collected from elytra), in coll. BPI; Alaska, Matanuska Valley, 29 Jun. 1944, leg. J.C. Chamberlin, on *H. tredecimpunctata*, permanent slides D.Haelew. 179a (five specimens collected from right metacoxa), 179b (seven specimens collected from

sternum II), 179c (eight specimens collected from sternum III to IV), in coll. BPI; North Dakota, Grant County, Lake Tschida (Heart Butte Reservoir), 21 May 1966, leg. R.D. Gordon, on *H. tredecimpunctata*, permanent slides D.Haelew. 180a (twenty specimens collected from right elytron), 180b (specimens collected from left elytron), in coll. BPI. UK, Oxfordshire, Sutton Courtenay, 10 Nov. 2011, leg. R.F. Comont, on *H. axyridis*, permanent slide FH 00313261 (five specimens collected from left elytron). The Netherlands, North Brabant, Tilburg, Nov. 2011, leg. B. Horvers, on *H. axyridis*, permanent slide FH 00313239 (over ten specimens collected from different body parts). BELGIUM, East Flanders, Destelbergen, town of Heusden, N51°0'21.52" E3°48'16.42", 26 Jun. 2013, leg. D. Haelewaters, on *H. axyridis*, permanent slide FH 00313262 (four specimens collected from third sternite).

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